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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF SOILS—BULLETIN No. 83.

MILTON WHITNEY, Chief of Bureau.

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# A BENEFICIAL ORGANIC CONSTITUENT OF SOILS: CREATININE.

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INTRODUCTION: IMPORTANCE OF BENEFICIAL SOIL  
CONSTITUENTS. OSWALD SCHREINER, Scientist in Charge  
Fertility Investigations.

I. THE ISOLATION OF CREATININE FROM SOILS.

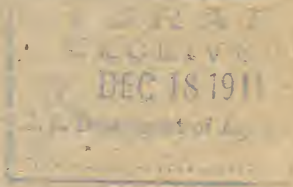
EDMUND C. SHOREY, Scientist in Fertility Investigations. 11-25

II. THE ORIGIN OF CREATININE IN SOILS.

M. X. SULLIVAN, Scientist in Fertility Investigations.

III. EFFECTS OF CREATININE ON GROWTH AND AB-  
SORPTION.

J. J. SKINNER, Scientist in Fertility Investigations.





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## LETTER OF TRANSMITTAL.

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U. S. DEPARTMENT OF AGRICULTURE,  
BUREAU OF SOILS,  
*Washington, D. C., July 10, 1911.*

SIR: I have the honor to transmit herewith the manuscript of a scientific paper entitled "A Beneficial Organic Constituent of Soils: Creatinine." This paper is the result of a study of the organic matter of the soil by a number of the soil scientists of this bureau.

The results of this investigation throw much light upon the problems of soil fertility and I therefore recommend their publication as Bulletin No. 83 of the Bureau of Soils.

Respectfully,

MILTON WHITNEY,  
*Chief of Bureau.*

Hon. JAMES WILSON,  
*Secretary of Agriculture.*





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## IMPORTANCE OF BENEFICIAL SOIL CONSTITUENTS.

By OSWALD SCHREINER,  
*Scientist in Charge, Fertility Investigations.*

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There have appeared a number of bulletins from this laboratory dealing with the nature and properties of soil organic matter. The extended study of this subject, about which so little definite information existed in chemical and agricultural literature, was undertaken as a result of the discovery of a harmful property in extracts from unproductive soils which was shown to be organic in character rather than inorganic. An investigational survey of organic compounds likely to occur in soils showed that some of these had decided effects on plant growth, and an examination of the organic matter of unproductive soils led to the discovery and isolation of several harmful constituents. The details of these investigations, as well as the effect of such substances in hindering plant growth, interfering with the normal processes of plant life and plant metabolism, have been given in the earlier reports of these investigations. All of these facts showed the great importance of more accurate knowledge concerning that most important and yet least understood soil constituent, the organic matter or humus of soils. A thorough inquiry into the nature of this material has been made in this laboratory and already a considerable fund of definite information is at hand. Starting without methods and with no definite organic soil constituent ever isolated from soil, the work has steadily progressed until to-day over 25 organic soil constituents have been isolated, definitely identified, and classified. The list of the compounds so far isolated varies from simple compounds, containing only carbon and hydrogen, to complex compounds, containing carbon, hydrogen, oxygen, nitrogen, and phosphorus. They are compounds which exist as such in living plants or arise from the decay or decomposition of the proteins, fats, nucleic acids, carbohydrates, and other plant and animal substances, as the result of purely chemical oxidations and reductions and through the intervention of molds, bacteria, protozoa, and other biological influences in soils. According to the factors operative in the decay of this plant and animal debris, the course of change will be different, resulting under one set of conditions in the formation of the abnormal and harmful constituents alluded to above and under other conditions in the formation of normal or beneficial organic constituents of soils.

It is an essential part of this survey of the organic matter in soils to learn the properties of the individual constituents and their effect on the life within the soil and on growing crops. Owing to lack of material and the difficulty of getting large amounts of the constituents in question, this inquiry is naturally a difficult and tedious one and consequently the determination of the effect on crop growth of all of the constituents isolated has not yet been feasible. So far as one of the constituents is concerned, this has been quite thoroughly done and the report made in a former bulletin. In the present bulletin the presence in soils of a constituent decidedly beneficial to growing crops will be emphasized. This soil constituent is creatinine, a nitrogenous compound, the isolation, identification, and chemical properties of which will be given in the first of the following papers, its occurrence in plants and seeds, and especially the fact that it can be found in soil and water in which plants are growing is presented in the second paper, and the effect of this compound on plant growth and its ability to serve as a direct source of nitrogen to the plant, in the third paper.

The three facts demonstrated by these investigations are of fundamental significance in soil fertility. The recognition of this directly beneficial soil constituent is no less important than the recognition that harmful soil constituents exist. The organic matter of soils has been considered almost wholly as having an indirect effect in agriculture, to be as it were merely a source for important elements like phosphorus and nitrogen. These researches show individual constituents to have decided effects by themselves in hindering or helping plant growth. This nitrogenous constituent appears to be as favorable as soil nitrates to crop production and even to be able to replace the latter in such a manner that the amount of nitrate absorbed is materially lessened in its presence while plant growth is increased. The amount in soils is, moreover, comparable with the amount of nitrates as usually found in agricultural soils. Creatinine is, however, only one of a long list of nitrogenous and phosphorus-carrying organic compounds of the soil and the remarks here made will probably find application to many other of these soil constituents.

The most beneficial manures under normal circumstances are those of organic origin, and the presence of such directly beneficial compounds, like creatinine, in well-rotted stable manure and in green manures, like cowpeas, goes far toward explaining why these manures are more beneficial to soil as a rule than are equivalent parts of fertilizer in the purely mineral forms.

The chief aim in the agricultural use of nitrogen is to convert this into nitrates by chemical and biological means, an operation far from simple, especially by distinctly chemical processes. The present

researches are very suggestive of the fact that for agricultural purposes it may not be necessary to convert all nitrogen into nitrates, but that nitrogen of waste nitrogeneous materials in the industries can be converted into compounds of the nature here discussed and so make available to agriculture much nitrogen now lost because of the difficulty to convert it into nitrates. These, however, are matters for further investigation. The present bulletin will content itself with demonstrating that a nitrogenous constituent, creatinine, exists in soils, in manures, and in many plants and seeds, whereas hitherto it was recognized only in connection with products of animal origin; that it is beneficial to crops, and that it seems to be able to replace nitrates in aiding plant growth.





# THE ISOLATION OF CREATININE FROM SOILS.

By EDMUND C. SHOREY,  
*Scientist in Fertility Investigations.*

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## DISCOVERY IN SOILS.

In the course of investigations into the nature of the organic constituents of soils a crystalline organic compound was isolated and identified as creatinine.

Reactions indicating the presence of this compound were first observed in a solution of a portion of the soil organic matter obtained in the following manner: The soil was treated with 2 per cent sodium hydroxide solution for half an hour, and then without separation of the alkaline extract from the soil, acetic acid in slight excess was added and the acid solution filtered from the soil and precipitate formed. The acid filtrate was neutralized with sodium hydroxide and, without filtering, a solution of lead acetate added and the precipitate formed was separated by filtration. On the addition of ammonia to the filtrate a further precipitate was produced which was filtered off, washed, and decomposed by hydrogen sulphide. The filtrate from lead sulphide was concentrated to a small volume and was found to give the Jaffé, Weyl, and Salkowski color reactions for creatinine.

It was further found that if the filtrate from the neutral lead acetate precipitate was made alkaline with sodium hydroxide instead of ammonia, and the resulting precipitate decomposed and concentrated, the color reactions were obtained more strongly, indicating a more complete precipitation of the creatinine by this means.

From the known behavior of creatinine when treated with lead acetate in alkaline solution it was evident that neither of the methods of precipitation mentioned would result in complete separation of the creatinine present, and for the isolation of this compound recourse was had to other methods.

## METHODS OF ISOLATION FROM SOILS.

### ALKALINE EXTRACTION.

The method by which creatinine was first isolated from soils, and already noted elsewhere,<sup>1</sup> was to apply to an alkaline soil extract a method recommended by Balke<sup>2</sup> for the separation of purine bases,

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<sup>1</sup> Science, **33**, 340 (1911).

<sup>2</sup> Jour. prakt. Chem. (2), **47**, 537 (1893).



and used for that purpose in this laboratory.<sup>1</sup> This method depends on the fact that when there is precipitation of cuprous oxide from Fehling's solution by a reducing body, purine bases and some related compounds combine with the cuprous oxide and are found in the precipitate. Creatinine behaves in this way, as was first pointed out by Masche.<sup>2</sup>

The method as applied to the isolation of creatinine from soils was carried out as follows: An alkaline extract of the soil, made by treatment for a short time with 2 per cent sodium hydroxide, was made exactly neutral with acetic or sulphuric acid and filtered. The neutral filtrate was heated to boiling and a little dextrose added and then Fehling's solution, slightly in excess of that required by the dextrose present. The precipitate formed was separated by filtration, well washed, and decomposed by hydrogen sulphide. The filtrate from the copper sulphide was concentrated to a small volume under reduced pressure, a small quantity of a concentrated solution of zinc chloride and a little sodium acetate added, and the whole allowed to stand several days. Within a few hours crystals began to form, and in 48 hours these were observed to have the characteristic appearance of creatinine zinc chloride. The crystals were separated from the mother liquor by filtration or, when the quantity of material was very small, by placing the whole mass on a porous plate. After separation the crystals were washed with a little cold water, suspended in water and boiled with some freshly precipitated lead hydroxide, filtered, and the filtrate concentrated to a small volume; on standing a short time crystals formed having the appearance, solubility, and color reactions of creatinine.

#### ALCOHOL EXTRACTION.

From another portion of the same soil from which creatinine had been obtained by the method just outlined, creatinine was also obtained by alcoholic extraction. About 100 grams of air-dried soil were extracted in a Soxhlet extractor for seven hours with 95 per cent alcohol. The alcohol was removed from the colored extract so obtained by evaporation, water being added to keep the volume constant. The aqueous solution was filtered from resinous and fatty matter and evaporated to a small volume under reduced pressure. The concentrated solution gave all the color reactions for creatinine and on treatment with zinc chloride gave, after standing two days, crystals of the characteristic creatinine zinc chloride.

<sup>1</sup> Chemical Nature of Soil Organic Matter. By Oswald Schreiner and Edmund C. Shorey. Bul. 74, Bureau of Soils, p. 41 (1910); Jour. Biol. Chem., 8, 391 (1910).

<sup>2</sup> Zeit. anal. Chem., 1878, p. 134.

## AQUEOUS EXTRACT.

Creatinine was also obtained from the same soil by simple extraction with water. About 5 kilograms of soil were extracted with cold distilled water in a percolator until 8 liters of extract had been obtained. This extract was concentrated to about 100 cubic centimeters by evaporation under reduced pressure, and filtered from a small quantity of insoluble matter present. The solution so obtained gave all the color reactions for creatinine, and on treatment with zinc chloride, crystals of the characteristic creatinine zinc chloride were formed. From these, by treatment with lead hydroxide in the manner already described, crystalline creatinine was obtained.

## THE CHEMISTRY OF CREATININE.

## OCCURRENCE AND PROPERTIES.

Creatinine,  $C_4H_7ON_3$ , is found normally in human urine, and has been reported as found in a number of fluids and tissues for the most part of animal origin; in dogs' urine,<sup>1</sup> in the flesh of certain fish,<sup>2</sup> in the muscles of mammalia,<sup>3</sup> in beef extract,<sup>4</sup> in crab extract,<sup>5</sup> and among the products of certain bacteria.<sup>6</sup>

Its occurrence in plants, demonstrated in another section of this bulletin, has not heretofore been noted, and its occurrence as the result of bacterial growth has been noted but a few times.

Creatinine is the anhydride of creatine or methyl guanidine acetic

acid  $NH:C \begin{array}{l} \swarrow NH_2 \\ \searrow N(CH_3)CH_2 \end{array} \begin{array}{l} COOH \\ | \\ NH-CO \\ | \\ N(CH_3)CH_2 \end{array}$  and is represented structurally by the formula  $NH:C \begin{array}{l} \swarrow NH \\ \searrow N(CH_3)CH_2 \end{array} \begin{array}{l} CO \\ | \\ N(CH_3)CH_2 \end{array}$ . It is readily transformed into creatine by

heating with acids or, as Folin and Denis have recently shown, by heating it with its water of crystallization under pressure.<sup>7</sup>

Creatinine is sparingly soluble in cold water and in alcohol and is insoluble in ether. It is easily soluble in hot water, and crystallizes from a hot saturated solution in monoclinic plates or prisms without water of crystallization. From a cold saturated solution it crystallizes in large plates containing two molecules of water. It forms crystalline salts with mineral acids and double salts with many mineral salts. The double salt with zinc chloride and hydrochloric

<sup>1</sup> Voit, *Jahr. Fortsch. Chem.*, 1867, 792.

<sup>2</sup> Krukenberg, *Jahr. Fortsch. Thier. Chem.*, 1881, 344.

<sup>3</sup> Monari, *Gazz. chim. ital.* 17, 382 (1888).

<sup>4</sup> Kutscher, *Zeit Nahr Genussmittl.*, 10, 528 (1905); 11, 582 (1906).

<sup>5</sup> Ackerman & Kutscher, *Untersuch Nahr Genussmittl.*, 13, 183 (1907).

<sup>6</sup> Zinno, *Riforma Medica*, 9 (3), 806 (1893); Antanoff, *Central. Bakt. Parasit*, 43, 209 (1907).

<sup>7</sup> *Jour. Biol. Cher.*, 8, 300 (1910).

acid  $(C_4H_7ON_3HCl)_2 ZnCl_2$  is easily soluble in water and alcohol, but if to a solution of this salt sufficient sodium acetate be added to neutralize the hydrochloric acid the salt  $(C_4H_7ON_3)_2 ZnCl_2$  is formed, and, being little soluble in water, is precipitated. The crystalline form of this salt is characteristic, being in the form of fine needles arranged in the form of balls or tufts showing a radiating structure, or when pure and in sufficient quantity, in the form of monoclinic plates. It is almost insoluble in alcohol, and has been used for the preparation of creatinine, and its characteristic crystalline form affords, when obtained, conclusive evidence of the presence of creatinine.

#### COLOR REACTIONS.

Creatinine in water solution gives a number of color reactions that have been depended on largely as indicating or proving the presence of this compound in fluids such as urine.

*Jaffé's reaction.*—If a solution of creatinine be made alkaline with sodium hydroxide and then a few drops of picric acid solution added, a red color is formed.<sup>1</sup> The color, which is similar to that of a concentrated solution of potassium dichromate, becomes orange on dilution. A quantitative colorimetric method for determining creatinine based on this reaction has been devised by Folin<sup>2</sup> and has been almost universally adopted in determining the quantity of this compound in urine. In the use of this reaction for either qualitative or quantitative purposes the absence of other compounds that give a similar color or that give a color that would obscure the one given by creatinine must of course be assured. In urine the only compound of this nature likely to be present is acetone, the presence of which can of course be easily determined. In solutions or extracts, however, containing soil organic matter there is the possibility of the presence of other compounds that may simulate or obscure the color given by creatinine, but whose presence can not be definitely determined, because of lack of efficient methods. Levulinic acid and furfural both give the Jaffé reaction strongly, and while neither have been definitely identified as present in soils, there are some indications of the presence of the former, and the latter might very easily be present or formed in soil extracts at some stage in the operation.

*Weyl's test.*—A watery solution of creatinine to which a small quantity of a solution of sodium nitroprusside has been added gives on the addition of sodium hydroxide a red color which soon changes to yellow.<sup>3</sup> As with the Jaffé reaction, the presence of certain other compounds may interfere with the use of this test either by giving a color that obscures the one given by the test, or giving a similar one.

<sup>1</sup> Zeit. physiol. Chem., 10, 399 (1896).

<sup>2</sup> Zeit. physiol. Chem., 41, 223 (1904).

<sup>3</sup> Ber. chem. Ges., 11, 2175 (1878). Arnold, Zeit. physiol. Chem., 49, 397 (1906).



Levulinic acid gives under the conditions of this test a color similar to that given by creatinine, and this test with others has been used to establish the presence of levulinic acid in the decomposition products of nucleic acid.<sup>1</sup> Furfural and the products resulting from heating pentose-yielding material with acid give a similar color, although in no case is the fading of the red color to yellow so pronounced as with creatinine. The dark-purple color given by sulphides in the presence of sodium nitroprusside and sodium hydroxide would of course obscure the color given by creatinine, and, as was noted in the case of Jaffé's reaction, there are probably in soil extracts unknown compounds that give either a similar color or one that would obscure the one given by creatinine.

*Salkowski's reaction.*—If the yellow solution resulting from Weyl's test be acidified with acetic acid and then heated, the solution turns green, then blue, and Prussian blue is precipitated if much creatinine is present.<sup>2</sup> Both levulinic acid and furfural give the final blue color and precipitate, but in the case of levulinic acid the solution after the addition of acetic acid and before heating is purple. The reagents alone will give the final blue color and precipitate if the solution before acidifying be allowed to become warm.

Since soil extracts or solutions prepared from them may contain compounds that are known to give color reactions similar to those given by creatinine, and since there is the possibility of the presence of others as yet unknown, it is evident that results obtained with the color tests usually considered indicative of the presence of creatinine can not be considered conclusive proof of the presence of this compound in the soils from which the soil extracts were prepared.

#### METHODS OF IDENTIFICATION.

When indications of the presence of creatinine are obtained by any of the color tests just described, this can best be confirmed and established by the preparation of the creatinine zinc chloride ( $C_4H_7ON_3$ )<sub>2</sub>ZnCl<sub>2</sub>. This salt, almost insoluble in alcohol and difficultly soluble in water, is formed when concentrated solutions of creatinine and zinc chloride are brought together in the absence of free mineral acids, a condition usually obtained by the addition of a little sodium acetate. When any large quantity of creatinine is treated in this way there is immediate precipitation, but when small quantities are being dealt with precipitation does not begin for several hours and is not complete for several days. The crystals of this salt are quite characteristic in form, although this form is so modified by the concentration of the solution, the presence of other bodies, and other conditions that they appear to differ widely. A study of the

<sup>1</sup> Inouye, *Zeit. physiol. Chem.*, **42**, 116 (1904).

<sup>2</sup> *Zeit. physiol. Chem.*, **4**, 133 (1880); **9**, 127 (1885).

form assumed when the crystals first appear, and of their growth, and of the form assumed on recrystallizing, soon results in familiarity with the characteristic appearance, so that the compound can be readily identified, no matter what form it may assume. Usually it appears in balls with radiating structure, due to their being made up of fine needles. Under other conditions its first appearance may be in starlike plates which eventually assume the form of bunches of radiating needles or plates. If the crystallization is slow, the plates may grow to a well-developed form. Under still other conditions the radiating needles are bunched in tufts rather than balls. This last form is the one assumed by the pure compound when a concentrated solution is cooled rapidly. Some of the forms are shown in the photomicrographs of Plate I.

When sufficient creatinine zinc chloride can be prepared pure, its identification by method of preparation, solubility, and crystalline form may be supplemented by analysis, although once familiarity with the characteristic behavior and appearance of this compound is acquired, this is not essential. A preparation of creatinine zinc chloride made from an alkaline soil extract in the manner already described was purified by recrystallization, dried on a porous plate, and then in a desiccator, and nitrogen and zinc oxide determined in the following manner: A portion of the creatinine zinc chloride, 0.36 gram, was digested with a small quantity of sulphuric acid and a little potassium sulphate, as in the Gunning modification of the Kjeldahl method for total nitrogen. The resulting solution was made alkaline with sodium hydroxide and distilled into standard acid in the usual manner. After the removal of the ammonia by distillation the solution was made acid with acetic acid and the zinc in solution precipitated by hydrogen sulphide, the zinc sulphide collected, the precipitate and filter paper treated with nitric acid, dried, and carefully ignited and weighed as zinc oxide. The analysis gave the following figures:

|       | Found. | Calculated for<br>(C <sub>4</sub> H <sub>7</sub> ON <sub>3</sub> ) <sub>2</sub> ZnCl <sub>2</sub> . |
|-------|--------|-----------------------------------------------------------------------------------------------------|
| N.... | 23.22  | 23.16                                                                                               |
| ZnO . | 22.31  | 22.45                                                                                               |

The formation of free creatinine from creatinine zinc chloride by boiling with lead hydroxide in the manner already described, the crystallization of the creatinine so obtained, and observations regarding its crystalline appearance, solubility, and color reactions, may be resorted to in further confirmation of the identity of the compound. The crystalline appearance of creatinine, monoclinic plates or prisms, one form of which is shown in the photomicrograph of Plate I, is, however, not characteristic enough to form the basis of identification.

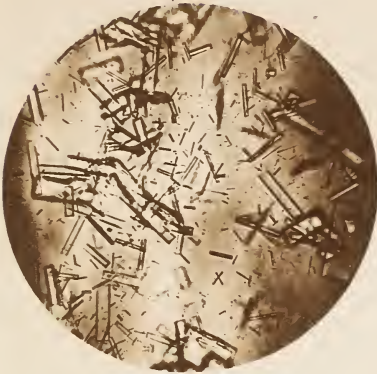


FIG.1 - CREATININE

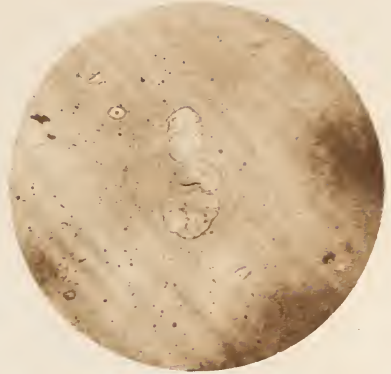


FIG.2- CREATININE ZINC CHLORIDE (CONCRETIONS)

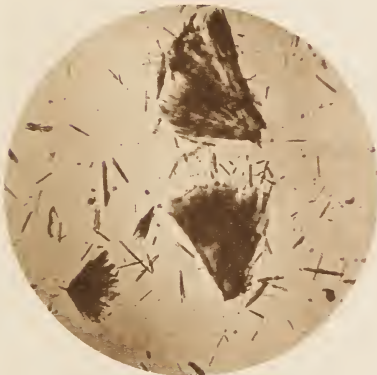


FIG.3- CREATININE ZINC CHLORIDE (TUFTS)



FIG.4- CREATININE ZINC CHLORIDE (PLATES)

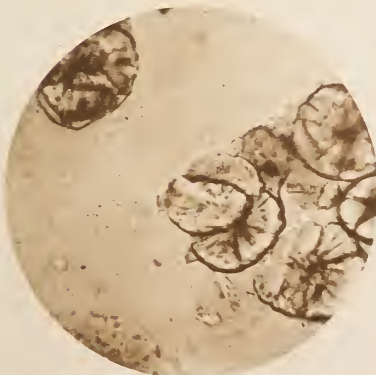


FIG.5- CREATININE ZINC CHLORIDE FROM CREATININE OF WHEAT



FIG.6- CREATININE ZINC CHLORIDE FROM CREATININE OF CLOVER

PHOTOMICROGRAPHS OF CREATININE FROM SOILS (FIG.1), CREATININE ZINC CHLORIDE PREPARED FROM IT (FIGS.2,3,AND 4),AND CREATININE ZINC CHLORIDE PREPARED FROM THE CREATININE OF WHEAT AND CLOVER (FIGS.5 AND 6).







WHEAT PLANTS GROWING IN CULTURE SOLUTIONS WITH AND WITHOUT CREATININE, AND CONTAINING VARIOUS PROPORTIONS OF POTASH AND PHOSPHATE BUT NO NITRATE.





## EXISTENCE OF CREATININE IN SOILS.

At this point the question naturally arises whether the isolation of creatinine by the methods described indicates or proves the presence of creatinine as such in the soil. In deciding this question in the case of other organic compounds isolated from soils the conclusion that the compounds in question must be in the soil as such was based on the fact that all the knowledge of the behavior of the compounds and their antecedents indicated that they could not be formed by any of the treatments to which the soil or soil extracts had been subjected. In the case of creatinine the question is complicated; first, by the fact that nothing is definitely known regarding its antecedents, i. e., what complex molecule, if any, it is derived from, no matter where found; and, second, by the fact already mentioned that creatinine is readily changed to creatine and vice versa according to the conditions imposed. Consequently, from general considerations only, it might seem that the creatinine isolated by methods which involve chemical reagents and heat might be derived from creatine or some unknown more complex body from which creatinine was easily split off. More detailed consideration, however, of the methods involved effectually disposes of the possibility that the creatinine is wholly derived from creatine. While the transformation of creatine and creatinine back and forth into each other is comparatively easy, Folin has shown<sup>1</sup> that it is not so easy as many investigators have assumed. All the operations in the methods used except the final concentration were such as would result in the change of creatinine back to creatine rather than otherwise. The alkaline extraction and boiling with Fehling's solution would no doubt bring about this change to some extent so that the creatinine finally obtained was that which had resisted this treatment. The final concentration of the aqueous solution at a low temperature might form some creatinine from creatine, but investigation has shown that this change could be but slight and could never involve the whole of the creatine if it were present. Treatment of these solutions by boiling for several hours with acid resulted in but slightly increased colorimetric readings for creatinine. In other words, the small quantity of creatine present shown by this method was out of all proportion to what would remain after evaporation under reduced pressure if creatine only had been present in the original solution.

The question of the possibility of the creatinine obtained being formed during the treatment from some more complex antecedent easily broken down unfortunately can not be disposed of until some definite information regarding such a complex, if there is one, is available.

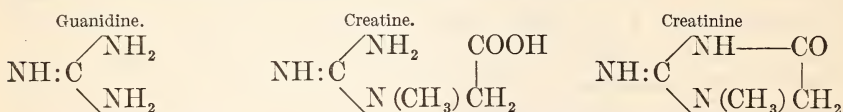
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<sup>1</sup> The Chemistry and Biochemistry of Creatine and Creatinine. Festschrift Olof Hammarsten, III.

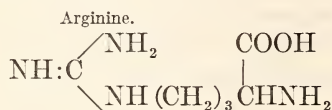
In the light of all the knowledge available and on consideration of the bearing of the methods on the final product it seems safe to conclude that a considerable portion at least and probably all of the creatinine isolated from soils is present in the soils as such.

#### RELATION OF CREATININE TO OTHER COMPOUNDS.

With the question of the possible antecedent of creatinine in mind it may be well at this point to consider its relation structurally to other compounds. Creatinine, as has been stated, is the anhydride of creatine or methyl guanidine acetic acid, and the relation of guanidine, creatine, and creatinine is shown as follows:

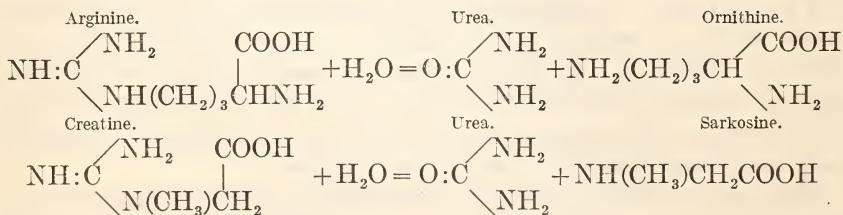


Arginine, a compound that occurs in plants and animals and which has been found in soils, is guanidine  $\alpha$ -amino normal valerianic acid, and its relation structurally to the compounds just mentioned is shown by the formula:



Guanidine has been found in plants<sup>1</sup> and in the autodigestion of certain animal organs,<sup>2</sup> and the relationship evidently existing between this simple compound and the more complex arginine and of both to creatinine is of interest and suggestive of a common origin.

The close relationship between arginine and creatine and consequently of creatinine is shown by their behavior when heated with barium hydroxide. Under these conditions arginine decomposes into urea and ornithine, while creatine gives urea and sarkosine or methyl glycocoll:



The same relationship is shown in the synthesis of both compounds. Creatine has been synthesized from sarkosine and cyanamid,<sup>3</sup> while

<sup>1</sup> Schulze, Ber. chem. Ges. **25**, 658 (1893).

<sup>2</sup> Otori, Zeit. physiol. Chem., **42**, 453 (1893).

<sup>3</sup> Stecker, Jahr. Fortsch. Chem., 1868, 686.

the synthesis of arginine has been brought about by the action of cyanamid on ornithine.<sup>1</sup>

#### RELATION OF CREATININE TO OTHER ORGANIC SOIL CONSTITUENTS.

Of the two compounds just mentioned as being related to creatinine structurally, arginine only has been found in soils and only infrequently and in very small quantities. Since arginine is a constant decomposition product of protein it must enter into the organic material added to soils, and the evidence at hand indicates that it soon disappears as such. Guanidine, although it has been found in plants, does not seem to be a usual soil constituent, and although it has been looked for in a number of soils it has not yet been found. The purine bases bear a relation to guanidine and through it to creatinine, and it is worthy of note that guanine, the only commonly occurring purine base up to the present not found in soils, yields guanidine on oxidation. Guanine is, however, a constituent of organic fertilizers such as guano. The possible connection of creatinine with this purine group, while of interest, is in the present state of knowledge, however, purely speculative.

The possible relation of creatinine to more complex bodies found in the soil or added to soil in dead vegetation is still more obscure, but a few observations made in this connection are worthy of record. Nucleic acids of unknown constitution have been found in several soils.<sup>2</sup> Both nucleic acid from soil and yeast nucleic acid prepared by Merck have been found to give the color reactions for creatinine after heating or even warming for a few minutes with dilute hydrochloric acid. From a solution of yeast nucleic acid treated in this way crystalline creatinine zinc chloride was prepared. It was found, however, that on washing the nucleic acid with cold dilute hydrochloric acid the nucleic acid no longer had the property of giving creatinine on heating with acid. The hydrochloric acid washings, however, after heating showed the creatinine reactions. Whether the explanation of this lies in the inclusion of some creatine in the nucleic acid or whether creatinine is actually split off from the nucleic acid or some complex included with it is as yet unknown. Phytin, or oxymethylene diphosphoric acid anhydride, is an organic compound that occurs in the seeds of many plants and must find its way into the soil. So far, this compound has not been isolated from any soil, but it was found that a crude preparation prepared from wheat bran gave on heating with hydrochloric acid a solution which gave the color reactions of creatinine, and from this solution crystals of creatinine zinc chloride were prepared. On purifying the phytin, however, in the

<sup>1</sup> Schulze and Winterstein, *Ber. chem. Ges.*, **32**, 3191 (1899); *Zeit. physiol. Chem.*, **34**, 128 (1905).

<sup>2</sup> These researches on nucleic acid in soils will be reported more fully later.



usual way by precipitating it several times as the barium salt<sup>1</sup> it likewise no longer had this property.

#### PRESENCE OF CREATININE IN MANURES.

The relation of creatinine to plants and plant products generally is treated in a subsequent section of this bulletin, and the only further observations that will be made here bearing on the relation of creatinine to soil organic matter has to do with that added in agricultural practice, viz, organic manures. Creatinine has been found both in stable manure and in fresh cowpea vines as used in green manuring. A sample of well-rotted stable manure was extracted with water and the solution allowed to stand until further fermentation had ceased. From this extract creatinine was isolated by precipitation with Fehling's solution in the same manner as from soil extracts. The resulting solution gave the color reactions for creatinine and crystals of both creatinine zinc chloride and creatinine were prepared from the material isolated. Green cowpea vines were crushed and extracted with cold alcohol, the alcohol evaporated at a low temperature, and water added to keep the volume constant and the solution filtered from insoluble matter. From this solution creatinine zinc chloride was prepared by precipitating the creatinine with Fehling's solution and subsequent treatment with zinc chloride and also by treating the concentrated aqueous solution directly with zinc chloride.

#### ESTIMATION OF CREATININE IN SOILS.

The work so far done on the quantitative determination of creatinine in soils is but preliminary and no definite statement regarding the quantity present in soils can be made other than that it is small and apparently but a small portion of the total organic matter. The following facts bearing on this question were established. It is much more easily extracted from soil by alcohol than by water. Usually enough could be obtained by extracting 100 grams of soil with alcohol to establish its identity, but to accomplish this by aqueous extraction several kilograms of soil were usually necessary. In spite of this fact, alcohol extraction does not offer an available method for quantitative determination, as is shown by the following experiment: One hundred grams of soil were extracted with alcohol until the concentrated extract no longer gave any reaction for creatinine. Ten milligrams of creatinine were then added and the soil extracted with alcohol again, and the creatinine determined colorimetrically in the extract obtained. After 14 hours of continuous extraction only three-tenths of a milligram of creatinine was found in solution, and a second extraction of seven hours gave an amount too small to be deter-

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<sup>1</sup> Patten and Hart, *Am. Chem. Jour.*, 31, 564 (1904).

mined. The color obtained was not greater after heating these extracts with acid, showing that the creatinine had not been extracted and changed to creatine. Since both water and alcohol extraction seemed unavailable as the basis of a method, attention was given to alkaline extraction. In attempting to carry this out quantitatively several difficulties are met. The complete extraction of the organic matter from soil with dilute alkali is slow and results in a very large volume of solution. Again this solution is dark colored and the use of a colorimetric method is out of the question unless there is removal of the color and concentration of the solution. It is possible that alkaline extraction and precipitation of the creatinine from the solution with Fehling's solution or in some other way will ultimately afford a means of determining this compound in soils, but so far these attempts have not given satisfactory figures.

The quantity of creatinine that has been found in soils by any of the methods tried, while small, and representing but a small portion of the organic matter, is by no means negligible, being usually several parts per million of soil and usually equal to and sometimes in excess of the quantity of nitrates normally present.

There is every indication that in no case was there complete extraction of the creatinine, and it is moreover possible that this organic soil constituent is a fluctuating quantity, being generated by bacterial or other biological agencies in the soil under certain conditions, and being changed or removed from the soil by growing plants under other conditions.

#### DISTRIBUTION OF CREATININE IN SOILS.

The initial work on the isolation of creatinine from soils was done with a sample of Volusia silt loam from New York. The sample was from a field that had been in cultivation for many years. At the time the sample was taken and for several years previous the crops on the field had been very poor. So far as could be ascertained the field had never had any application of either commercial fertilizer or stable manure.

After the identity of the compound isolated from the Volusia silt loam was established as creatinine a number of other samples of soil were examined for the presence of this compound. Comparatively few soils have been examined, but so far no soil has been found in which the presence of creatinine was not indicated by the color reactions given by the extract.

Creatinine zinc chloride in addition to color reactions were obtained from samples of the following soils:

Frankstown stony loam from Pennsylvania. This sample was taken from a field that had been in cultivation 30 years, but had recently lain idle for 3 years. When put in cultivation again the crops were still very poor.



Clarksville silt loam from Kentucky. This sample was from an area where the crop yields were fair and the soil responded well to applications of stable manure.

Dunkirk clay from New York. Fair crop yields had been obtained on field where the sample was taken.

It would seem, then, that creatinine is at least a frequently occurring and probably a normal and constantly occurring constituent of soils. While the soils examined have all been under cultivation some years there is no theoretical reason for concluding that virgin soils differ from cultivated ones in this respect except perhaps in quantity.

#### SUMMARY.

In this section of this bulletin the isolation of a crystalline organic compound is described. This compound contains nitrogen and has been identified as creatinine.

Several methods by which the compound has been obtained from soils are described and the chemistry of creatinine and methods of identifying it are discussed.

The relation of creatinine to other organic compounds is pointed out and certain observations regarding the possible connection between creatinine and other organic soil constituents are made.

The occurrence of creatinine in organic fertilizers, such as stable manure and cowpea vines, that are added to soils in agricultural practice has been established, and it has been shown that creatinine occurs in soils of widely different type and from widely separated areas.

It is pointed out that while the quantity of creatinine in soils is small it is not negligible, being comparable to the quantity of nitrates normally present. It is further pointed out that the quantity of creatinine probably is, like the nitrates, a fluctuating quantity.

## THE ORIGIN OF CREATININE IN SOILS.

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In this paper are presented the results of experiments which throw some light on the question of the origin of creatinine in soils. The particular phase of the question studied is the presence of creatinine in plants and consequently in plant débris and the passage of the creatinine into the soil either by the disintegration of the plant débris or as a result of cell sloughing or direct passage from the living plant. Other possible origins of this soil constituent, such as its introduction in the animal excreta of stable manure or the formation by microorganisms, are not considered here.

Unless present in exceedingly small quantities creatinine gives certain color reactions. These reactions are: (1) Jaffé's reaction,<sup>1</sup> a red color with picric acid and a few drops of sodium hydroxide. With excess of alkali and on acidifying, the red color is discharged. (2) Weyl's reaction,<sup>2</sup> a ruby red color when a few drops of sodium nitroprusside are added to a creatinine solution and the mixture is made alkaline with sodium hydroxide. The red color soon turns yellow.<sup>3</sup> (3) Salkowski's reaction,<sup>3</sup> a greenish-blue color on adding an excess of acetic acid and warming the yellow solution obtained in Weyl's test. The greenish-blue color is in turn replaced by a blue color or a precipitate of Prussian blue.

The absence of these color reactions is a sure test that creatinine is absent or present in traces only. The occurrence of the color reactions, on the other hand, is but a presumptive test that creatinine is present, especially in the case of soil or plant extracts, since other substances show these reactions. When, however, no other substances which give these reactions are present in a solution, as in the urine, for example, the color reactions are decisive tests for creatinine and the Jaffé reaction has been made good use of as a quantitative test by Folin<sup>4</sup> and others.

The double salt of creatinine and zinc chloride is an important test for creatinine. It is formed best when zinc chloride and sodium acetate are added to a neutral or nearly neutral concentrated alcoholic

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<sup>1</sup> Zeit. physiol. Chem., **10**, 399 (1886).

<sup>2</sup> Zeit. physiol. Chem., **4**, 133 (1880); **9**, 127 (1885).

<sup>3</sup> Ber deutsch. chem. Ges., **11**, 2175 (1878).

<sup>4</sup> Zeit. physiol. Chem., **41**, 223 (1904).

solution of creatinine. The sodium acetate is added in sufficient quantity to neutralize any mineral acid that might be present since the characteristic double salt does not form in the presence of dilute mineral acids.

Though the literature pertaining to the biochemistry of the animal organism is filled with references to creatine and creatinine,<sup>1</sup> the possibility of the occurrence of these substances outside of animal products has not been investigated hitherto to any great extent. Zinno,<sup>2</sup> however, found that cultures of certain bacteria in peptone-salt solution gave Weyl's, Salkowski's, and Jaffé's reactions for creatinine, and he isolated the creatinine as creatinine zinc chloride. Later Antonoff,<sup>3</sup> relying merely on Weyl's reaction, reported the formation of creatinine by many bacteria in peptone-salt solution.

Prior to the present paper, however, creatinine has never been reported in vegetable matter, although it has been demonstrated often in the flesh of plant-eating animals.

In the present experiments the criteria for creatinine were: (1) The color reactions, especially Jaffé's and Salkowski's; (2) the formation of the characteristic double salt of creatinine and zinc chloride; (3) the regaining of creatinine from the zinc salt by boiling with lead hydroxide with a resulting solution which gave the color reaction

#### CREATININE IN PLANTED SOIL.

When the concentrated water extracts and water-glycerine extracts of a soil recently cropped in the greenhouse were boiled with Fehling's solution, there was more or less reduction of the copper with the formation of a greenish-white precipitate. The character of the precipitate suggested the presence of purine bases and creatinine in the soil solution, and it became of great interest to determine (1) whether or not the water and glycerine extracts of the cropped soils contained these compounds; (2) whether or not there was a relation between the content of these compounds in the soil and the growth of the crop. As regards the purine bases, it may be said in passing that by appropriate means their presence in the extracts was demonstrated. The main interest, however, was in creatinine for which the water and glycerine extracts of several soils recently cropped in the greenhouse were tested.

*Arlington clay loam.*—A sample of Arlington clay loam, upon which cowpeas had been grown for four years, was planted with wheat in paraffin wire pots and the cultures kept in the greenhouse for three weeks. Then the wheat seedlings were cut close to the soil.

<sup>1</sup> For review of literature see Magnus Levy, Van Noorden's *Handbuch der Pathologie des Stoffwechsels*, 1906, 1, 137; Hoogenhuyze and Verploegh, *Zeit. physiol. Chem.*, **46**, 415 (1905); Mel anby, *Jour. Physiol.*, **36**, 447 (1908); Shaffer, *Am. Jour. Physiol.*, **23**, 1 (1908).

<sup>2</sup> *Riforma Medica* (3), 9, 806 (1893).

<sup>3</sup> *Centr. Bakt. Parasitenk.*, **43**, 209 (1907).



The samples from the pots were combined and were sifted through a one-eighth inch mesh sieve. For the water extracts, 3 kilograms of the soil were extracted with 6 liters of water for 24 hours, with frequent stirring, especially in the early stages of extraction. For the glycerine extracts, 3 kilograms of soil were treated with 1,200 c. c. of pure glycerine for 12 hours, when 4,800 c. c. of water were added and the mixture stirred and allowed to stand 12 hours longer. The glycerine itself gave no creatinine test.

The water extract was filtered through paper and 2,500 c. c. were concentrated to a small bulk. The solution was then made slightly acid with hydrochloric acid and was treated with a large volume of alcohol. After a time a slight precipitate formed. The filtrate concentrated to about 20 c. c. gave Jaffé's and Salkowski's tests for creatinine. The glycerine extracts similarly concentrated to a small bulk gave stronger tests for creatinine. The concentrated glycerine extract of 2,500 c. c. of the original solution was sirupy from the glycerine. The sirupy concentrated glycerine extract was boiled with Fehling's solution and the mixture was filtered. The precipitate was suspended in water and freed from copper by means of hydrogen sulphide. The filtrate from the copper sulphide was concentrated as far as possible on the steam bath and was still found to contain some glycerine. To the concentrated solution, zinc chloride and sodium acetate were added with the formation of a white precipitate. In 24 hours, colorless rosettes of creatinine zinc chloride appeared.

*Dunkirk clay.*—About 8 kilograms of Dunkirk clay upon which wheat had grown for three weeks in the greenhouse were extracted for 20 hours with 6 liters of a 20 per cent glycerine solution. The supernatant liquid was poured off, the soil pressed in cloth in a press, and the combined solutions filtered through paper. The filtrate, concentrated to a small bulk, gave a strong Jaffé's reaction for creatinine. By the same procedure as used in the case of the Arlington clay loam above, the presence of creatinine was demonstrated by the formation of the creatinine zinc chloride.

*Dutchess silt loam.*—In the water extract of Dutchess silt loam and in the glycerine extract, creatinine was demonstrated in the same way by means of the double salt of zinc chloride and creatinine.

The creatinine zinc chloride crystals from the Arlington clay loam, the Dunkirk clay, and the Dutchess silt loam were boiled separately with freshly precipitated lead hydroxide. The filtrates were concentrated to a small bulk. All the concentrated solutions gave Jaffé's and Salkowski's tests. How much of this creatinine, which exists in the concentrated extracts in but small quantities, is present as such in the original extract can not be told, since in the process of concentrating some would be formed from creatine if present.

Creatinine was also found in the glycerine extract of the Arlington clay loam as it came from the field. As judged by the quantity of the double salt of creatinine zinc chloride obtained from the glycerine extracts of Arlington clay loam, planted and unplanted, similarly treated as regards concentrating, precipitating by Fehling's solution, freeing from copper by hydrogen sulphide, concentrating the filtrate from the sulphide and adding zinc chloride and sodium acetate, the extract of the soil planted and kept in the greenhouse for three weeks contained more creatinine than did the extract from the same soil unplanted used as a control. The increased amount of creatinine in the planted soil must have come (1) from an increased activity of micro-organisms on the plant débris and plant constituents and on the organic matter of the soil, or (2) directly from the growing plants.

#### CREATININE IN THE WATER IN WHICH SEEDLINGS HAD GROWN.

Since creatinine was found in the extract of soil on which wheat had grown for several weeks, it seemed possible that it might exist in water in which wheat seedlings had grown. Wheat was soaked for about five hours in water, and was then placed with a little water in an enameled pan to germinate over night. The seeds with the plumule just showing were then placed on a perforated aluminum disk which was floated on water by means of four air-tight glass tubes. In two days the water was changed. The water collected from six pans, about 12 liters in all, was concentrated on the steam bath to 300 c. c. To the concentrated solution a few cubic centimeters of concentrated hydrochloric acid and 1 liter of alcohol were added. In a short time a flocculent precipitate settled out. This precipitate gave a good pentose reaction with orcin and hydrochloric acid, contained phosphoric acid, and gave a slight xanthine test and probably contained nucleic acid. The filtrate from this precipitate was concentrated to about 25 c. c. and was then tested for creatinine by means of picric acid and sodium hydroxide. It may be said that many compounds, such as levulinic acid, tyrosine, pentose sugars, gallic acid, etc., which might be in the extract of plants, give the red color reaction with picric acid and sodium hydroxide, the red changing to yellow on adding acetic acid, so that this reaction was taken merely as a suggestion that creatinine was present in the water in which the seedlings were growing. The alcohol solution, which gave the color test, such as creatinine would give if present, was neutralized and brought to dryness, and was then treated with boiling absolute alcohol and filtered. The filtrate was again brought to dryness and the process of taking up with absolute alcohol and drying was repeated several times. The final slightly sirupy residue was treated with zinc chloride and sodium acetate in



the usual manner and was put in a desiccator. In a few days stars, radiating clusters of needles, and rosettes of creatinine zinc chloride appeared. To make a further test the mass containing the crystals was boiled with freshly prepared lead hydroxide and filtered. The filtrate, concentrated to a small volume, gave Jaffé's test for creatinine, and also Salkowski's test with the formation of Prussian blue. The original nonacidified solution, when strongly concentrated, likewise gave Salkowski's and Jaffé's tests for creatinine. The amount of creatinine in the water was, however, very small.

The water in which the seedlings were growing with merely the roots in contact with the water was next tested for creatinine. The water was changed every second day for 10 days. Each sample was concentrated as soon as collected and all were combined and brought to a small bulk in a vacuum drier. The concentrated solution was made slightly acid with hydrochloric acid and was then treated with four or five times its volume of 95 per cent alcohol. A slight flocculent precipitate formed. The filtrate was concentrated again in a vacuum to a small bulk and then gave positive results with Jaffé's and Salkowski's tests for creatinine. The solution was then brought to dryness either at room temperature or in the steam bath. The dry residue was taken up as far as possible with about 50 c. c. of absolute alcohol and the alcohol solution was brought to dryness. The residue was again taken up with alcohol and the process repeated as long as a residue insoluble in the alcohol was formed on drying. Finally the cold alcohol solution was concentrated to a small volume and zinc chloride and sodium acetate added in the customary way. In a few days typical stars and rosettes of the double salt of creatinine and zinc chloride appeared. Accordingly creatinine or some precursor of creatinine, such as creatine, must be in the water in which the roots were growing.

That most of the creatinine at times at least is not present as such in the culture water is shown by the fact that if the water without acidification be concentrated to a small volume in a vacuum, at a temperature between 50° and 60°, Jaffé's test is very slight and at times negative. By warming on the steam bath, especially in the presence of dilute hydrochloric acid, the creatine or other antecedent body is converted to creatinine.

Whether the presence of creatinine or creatine in the medium in which the plants are growing is to be attributed to the action of microorganisms on materials sloughed from the roots or to direct osmosis from the intact seed and roots is a question that is rather hard to settle definitely. The following experiment, however, shows that creatinine or creatine can pass from seeds by direct osmosis, and in the same way may pass through the roots in the normal growth of plants.

## CREATININE IN THE WATER IN WHICH SEEDS HAD BEEN SOAKING.

One kilogram of carefully sorted wheat seeds was placed in a flask with 2 liters of distilled water and the flask was kept in an ice box at 5° to 6° C. for five days. The liquid, to the quantity of about 1,500 c. c., was then poured off the slightly swollen, but entirely ungerminated seeds. In the liquid was a medley of organic substances, among which a small amount of creatinine was detected, as the following test shows:

Five hundred cubic centimeters of the slightly turbid, yellowish solution poured from the seeds was concentrated on the steam bath. To the concentrated solution, which had become brownish in color, a few cubic centimeters of hydrochloric acid were added, and then several volumes of 95 per cent alcohol. A slight flocculent precipitate formed. The filtrate was concentrated on the steam bath and made neutral by means of dilute sodium hydroxide. The neutralized solution gave Jaffé's and Salkowski's tests for creatinine. The solution was then allowed to dry in air. The dry residue was taken up with water and treated with lead acetate. The filtrate was freed from lead and was then brought to dryness on the steam bath. The dry residue was treated with absolute alcohol and the alcoholic solution again brought to dryness. The process of taking up with alcohol and drying was repeated until a residue was secured which was entirely soluble in absolute alcohol. The sirupy residue was treated with zinc chloride and sodium acetate and was placed in a desiccator. In about a week crystals of creatinine zinc chloride made their appearance in the brownish sirupy mixture. Boiled with lead hydroxide and filtered, the concentrated solution gave a good Jaffé test. On evaporating to dryness and taking up with absolute alcohol a solution was obtained which on concentrating and adding zinc chloride and sodium acetate gave the typical rosettes of creatinine zinc chloride. The creatinine must have come from the ungerminated seeds either as creatinine or as creatine or some other antecedent body which readily changes to creatinine.

## CREATININE IN VEGETABLE MATERIALS.

## WHEAT SEEDLINGS.

About 5,000 wheat seedlings, 9 to 11 days old, were ground and extracted for several hours with boiling alcohol. The hot alcohol was poured off and pressed out of the pulp and allowed to cool. The solution filtered through filter paper was concentrated to a small volume in a vacuum. The concentrated solution was filtered and treated with lead acetate to free it from coloring matter, etc. The fairly colorless filtrate was freed from lead by hydrogen sulphide. The filtrate from the lead sulphide was then concentrated to a small

volume. The concentrated solution gave Jaffé's test, i. e., a red color with picric acid and sodium hydroxide, which was discharged on adding excess of alkali, and also on acidifying. Accordingly, zinc chloride and sodium acetate were added to the solution. In the course of several days creatinine zinc chloride appeared in the mass, in the form of stars, crenated hexagonals, needle clusters, and rosettes. Treated with lead hydroxide in the customary manner, a solution was obtained which gave Jaffé's and Salkowski's tests for creatinine.

#### WHEAT SEEDS.

A pound of wheat seeds was crushed and extracted with cold water. The turbid solution was concentrated and filtered free of the precipitate which formed on boiling. Several volumes of 95 per cent alcohol were added to the solution and the mixture was filtered. The filtrate was freed from alcohol by distillation, and the residue from the distillate was further concentrated. This residue, which gave Jaffé's test for creatinine, was brought almost to dryness on the steam bath, and was then treated with a small volume of 95 per cent alcohol. The alcohol solution was brought to dryness, and the process of taking up with alcohol and drying was repeated several times. Finally, to the slightly brownish sirup soluble in cold alcohol, zinc chloride and sodium acetate were added. Typical crystals of the double salt of creatinine zinc chloride formed in the course of several days in the desiccator. By adding water to the mass much of the sirup dissolved and was poured off. The creatinine zinc chloride was left behind and showed on drying in a desiccator as practically colorless stars and rosettes. (See Pl. I, fig. 5.)

The double salt of creatinine zinc chloride was obtained also from the direct alcoholic extract of ground wheat seeds. The ground wheat seeds were extracted for several hours in a Soxhlet extraction apparatus. The hot alcohol extract was brought to dryness, taken up with a little cold water, filtered, and concentrated almost to dryness. The residue was then treated with absolute alcohol, and the alcohol soluble portion after being brought almost to dryness was treated with zinc chloride and sodium acetate. In a few days hexagonals, stars, and rosettes of needles were apparent under the microscope.

#### WHEAT BRAN.

Wheat bran was extracted in a Soxhlet apparatus with 95 per cent alcohol. The extract gave Jaffé's test for creatinine. The alcohol was evaporated off and the residue was treated with cold water and filtered. The filtrate was treated with lead acetate to remove coloring matter, etc. The filtrate from the lead precipitate was freed from lead by means of hydrogen sulphide and the practically



colorless filtrate was brought to dryness on the steam bath. The residue was then treated with absolute alcohol. The alcohol was again brought to dryness, etc., until the residue was all soluble in absolute alcohol. The addition of zinc chloride and sodium acetate gave rosettes of creatinine zinc chloride.

This experiment was repeated, using a large amount of bran. The sirupy mass containing the creatinine zinc chloride was placed on a porous plate and treated with alcohol and water until white. The residue on the plate was dissolved in boiling water and the solution was filtered. The filtrate was brought to dryness on the steam bath in a weighed porcelain dish. To the weighed residue nitric acid was then added and the solution was brought to dryness and carefully heated in a free flame until the nitric acid was all driven off. The residue was weighed as zinc oxide. The first weighing gave 22.6 per cent ZnO. On reheating, this was reduced to 21.8, which thereafter remained practically constant. Creatinine zinc chloride requires 22.4 per cent of ZnO.

#### WHEAT STRAW.

In alcoholic extracts of wheat straw practically no creatinine could be detected by the creatinine zinc chloride method. If the extracts, however, after being freed from coloring matter by lead acetate and from lead by hydrogen sulphide, were slightly acidified and boiled a fair amount of creatinine zinc chloride could be obtained. Apparently creatinine does not exist as such in the wheat straw while its congener creatine, or other antecedent body, does exist therein

#### WHEAT PROTEIN.

On boiling a water extract of ground wheat seeds a heavy precipitate formed. This precipitate, which gave protein color reactions, was washed with water, dilute hydrochloric acid, and alcohol until the washings on neutralizing and concentrating gave no tests for creatinine with picric acid and sodium hydroxide. The protein material was then boiled for 30 minutes with 3 per cent hydrochloric acid. From the filtrate, neutralized and concentrated and taken up with absolute alcohol, a solution was obtained which gave Jaffé's reaction and also crystals of creatinine zinc chloride on treating with zinc chloride and sodium acetate. Since the washings from the protein material also gave creatinine on heating with hydrochloric acid, the protein undoubtedly contained creatine.

Though there is some possibility that creatine and creatinine can be formed from the protein molecule or less complex nitrogen containing compounds, the origin of creatine and creatinine in the plant tissue is still an unsettled question.

Whatever is the origin of creatinine it exists in the wheat seed and in the wheat seedlings and is left more or less in the medium in which the seeds have germinated and the seedlings have grown.

#### RYE SEEDS.

Rye seeds ground to a coarse powder were extracted with hot 95 per cent alcohol. On cooling, the solution was filtered. The clear filtrate gave a strong Jaffé's test. Accordingly, it was concentrated to a very small volume and was tested for creatinine by zinc chloride and sodium acetate. The double salt of creatinine zinc chloride speedily formed in the mixture.

#### CLOVER AND ALFALFA SEEDS.

Alcohol extracts of clover and alfalfa seeds gave a strong creatinine color reaction with Jaffé's test. Since the alcohol extracts on cooling became somewhat turbid and also contained some coloring matter, water was added and the alcohol was driven off on the steam bath. To the water extract, lead acetate was added as long as a precipitate formed. The filtrate was freed from lead by hydrogen sulphide. The lead-free solution, brought almost to dryness on the steam bath, was treated with a small quantity of 95 per cent alcohol and filtered. The alcoholic solution was brought to dryness and the residue was treated with absolute alcohol. Finally, the part soluble in the absolute alcohol was concentrated to a small volume and was treated with zinc chloride and sodium acetate in the usual way. Creatinine zinc chloride in small quantities formed, both in the case of the clover extract and the alfalfa extract. Plate I, figure 6, shows the crystals of creatinine zinc chloride in the clover extract.

#### COWPEA PLANTS.

Creatinine was found in a similar way in the alcohol extract of young cowpeas, as shown in the previous section of this bulletin, and in mature cowpea plants.

#### POTATO TUBERS.

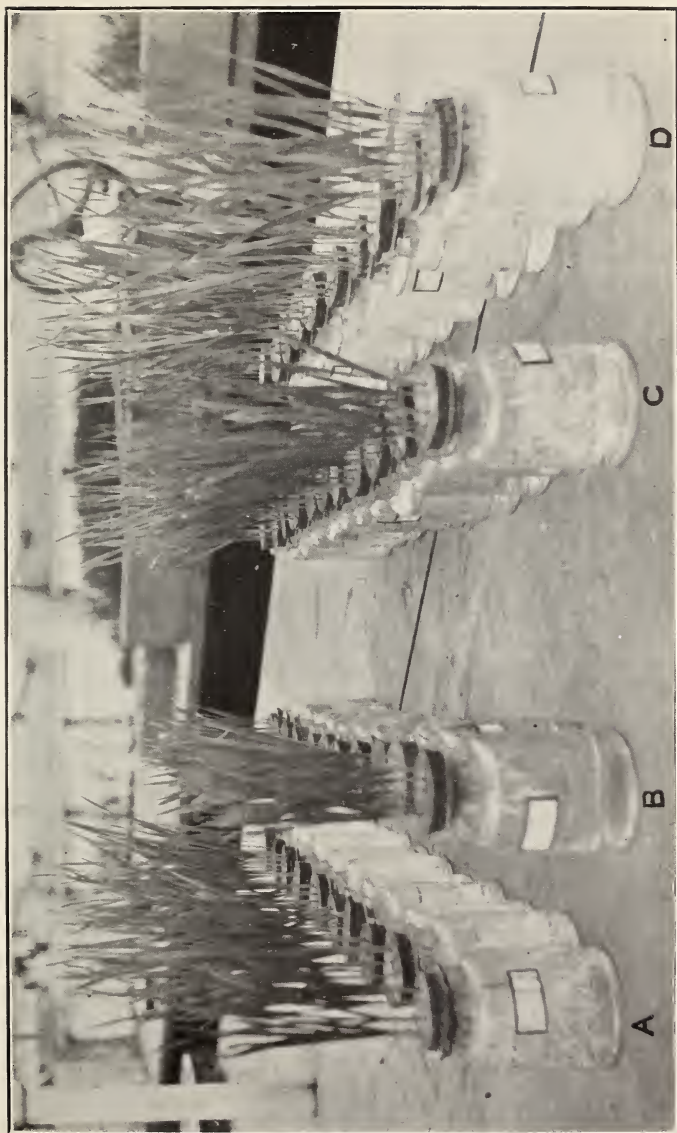
Potato tubers were ground to a fine pulp and the pulp was extracted with a liter of boiling alcohol. The filtered alcoholic extract was brought to dryness and the dry residue was taken up with cold water. The water solution was then treated with lead acetate and filtered. The filtrate was freed from lead and was concentrated to a small volume. The concentrated solution gave Jaffé's and Salkowski's tests for creatinine. The solution was brought to dryness and the residue taken up with absolute alcohol. To the absolute alcohol solution concentrated to a few cubic centimeters zinc chloride and sodium acetate were added. In the course of three or four days a few crystals of creatinine zinc chloride appeared in the mixture.



## SUMMARY.

Of samples of the same soil planted and unplanted kept side by side in the greenhouse, the planted soils gave larger amounts of creatinine. It would seem that the increase in the amount of creatinine was connected in some way with plant growth. Creatinine was also found in the water in which wheat seedlings had grown. The presence of creatinine in plants has not been previously reported. Various plants were tested for creatinine and its presence definitely ascertained. It was found in wheat seeds, wheat seedlings, and wheat bran, in rye, clover, alfalfa, cowpeas, and potatoes. If, as is suggested by several investigators, creatinine in the animal arises as the result of the breaking up of albumen, then it seems reasonable to expect that creatinine would be found in practically all plants.

Creatinine and creatine exist in vegetable matter to a slight degree. Though the amount of these compounds present is small it is worthy of attention, since by the decay of plants and by direct cell sloughing or even by osmosis the creatinine and creatine are left in water and soil where they exercise an effect on subsequent plant growth. Creatinine seems to persist for a considerable time in soils and may indeed increase in the soil by accumulation. Since both creatine and creatinine have a favorable effect on plant growth, their presence in plants and in the medium in which plants grow has considerable bearing on soil fertility.



WHEAT PLANTS GROWING IN CULTURE SOLUTIONS, WITH AND WITHOUT CREATININE, CONTAINING NO NITRATE, AND 8 PARTS PER MILLION OF  $\text{NH}_3$  AS NITRATE.

Series A. Culture solutions, composed of a fertilizer mixture, containing 8 parts per million  $\text{NH}_3$  as nitrate, composed of a fertilizer mixture, containing no nitrate, without creatinine. Series B. Culture solutions, composed of a fertilizer mixture, containing no nitrate, with creatinine. Series C. Culture solutions, composed of a fertilizer mixture containing 8 parts per million of nitrate, with creatinine.



## EFFECT OF CREATININE ON GROWTH AND ABSORPTION.

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In the first paper in this bulletin are reported the results of an investigation showing the presence in soils of creatinine, a nitrogenous, organic compound. In the second paper its occurrence in plants and in cropped soils is shown. The present paper embodies a series of experiments on the influence of creatinine on crops. The results of this investigation showing the effect of creatinine, it is believed, throw much light on the action of organic manures in soils, and the influence of soil organic matter on productivity.

### METHOD USED.

In studying the effect of creatinine on crops, wheat seedlings were grown in aqueous culture solutions containing the ordinary fertilizer salts, calcium acid phosphate, sodium nitrate, and potassium sulphate. Some of the cultures contained calcium acid phosphate only, some sodium nitrate only, and some potassium sulphate only. Other solutions were composed of mixtures of two salts, sodium nitrate and calcium acid phosphate, sodium nitrate and potassium sulphate, and calcium acid phosphate and potassium sulphate. Still other solutions had all three constituents in various proportions. The concentration of all the solutions was 80 parts per million of the fertilizer ingredients,  $P_2O_5$ ,  $NH_3$ , and  $K_2O$ . In cultures containing only one fertilizer salt, for instance calcium acid phosphate, the concentration was 80 parts per million of  $P_2O_5$ . If two salts were present, for instance calcium acid phosphate and sodium nitrate, the concentration was 80 parts per million of  $P_2O_5 + NH_3$ . If all three salts were present, the concentration was 80 parts per million of  $P_2O_5 + NH_3 + K_2O$ . The ratios of these constituents varied in 10 per cent stages. In all, there were 66 different cultures of nutrient solutions. Two sets of cultures were prepared; to one set were added merely the nutrient salts, to a similar set 50 parts per million of creatinine in each culture, in addition to the nutrient salts. The culture solutions were contained in wide-mouth bottles, holding 250 c. c., and 10 wheat seedlings grown in each culture, in a manner similar to that described in Bureau of Soils Bulletin 70. The culture solutions were changed every three days, four changes



being made in the course of the experiment. The solutions were analyzed for nitrates immediately after each change. The phosphate and potassium were determined on a composite solution of the four changes. Observations on the development of the plants were made during the experiment and photographs were taken. When the plants had grown for 12 days the green weight was recorded.

#### EFFECT OF CREATININE ON GROWTH.

Two sets of cultures composed of the fertilizer salts, calcium acid phosphate, sodium nitrate, and potassium sulphate in varying proportions, used singly and in combinations of two and three, were prepared and young wheat seedlings were grown in them from March 3 to March 15. To one set of cultures only the nutrient salts were added, to the second set 50 parts per million of creatinine were added to each culture. Every three days the solutions were changed and analyzed.

When the plants had grown for several days it was noticeable that the creatinine cultures were better developed, having broader leaves and longer and better developed roots. This was more noticeable in some of the fertilizer mixtures than in others.

The total growth made in the 66 cultures of nutrient salts without creatinine, designated as normal or control cultures, was 166.7 grams as against 181.2 grams in the case of the 66 cultures with 50 parts per million of creatinine. Putting the normal at 100, the latter becomes 109, or an increase of 9 per cent, as an average of the 66 cultures. The effect was much more pronounced in those fertilizer combinations containing no nitrogen and those low in nitrogen.

#### GROWTH IN CULTURES CONTAINING NO NITRATE, WITH AND WITHOUT CREATININE.

Table I gives the growth of two sets of cultures composed of mixtures of phosphate and potash, varying in 10 per cent stages, there being no nitrate in the solutions, the concentration being 80 parts per million of  $P_2O_5 + K_2O$  in each culture. To one set of cultures was added 50 parts per million of creatinine. In the fourth column are given the green weights of the cultures without creatinine, and in the last column are given the weights of the cultures with creatinine. It is apparent from these figures that the creatinine has caused a considerable increase in growth. This is true in each of the 11 cultures. For instance, in culture No. 3, which has 16 parts per million of phosphate and 64 parts per million of potash, the growth without creatinine was 1.950 grams and with 50 parts per million of creatinine it was 2.100 grams. Culture No. 6, which is composed of equal parts of phosphate and potash, produced 1.558 grams green weight when creatinine was absent and 2.408 grams when the creatinine was present in the solution. The growth in culture No. 9, which

is composed of 64 parts per million of phosphate and 16 parts per million of potash, was 1.444 grams without creatinine and 2.220 grams with creatinine.

TABLE I.—*Effect of creatinine on growth in cultures containing no nitrate.*

| No. | Fertilizer ingredients in culture solution. |                   |                   | Green weight of culture. |                  |
|-----|---------------------------------------------|-------------------|-------------------|--------------------------|------------------|
|     | P <sub>2</sub> O <sub>5</sub> .             | NH <sub>3</sub> . | K <sub>2</sub> O. | Without creatinine.      | With creatinine. |
|     | <i>P. p. m.</i>                             | <i>P. p. m.</i>   | <i>P. p. m.</i>   | <i>Grams.</i>            | <i>Grams.</i>    |
| 1   | 0                                           | 0                 | 80                | 1.400                    | 1.576            |
| 2   | 8                                           | 0                 | 72                | 1.470                    | 2.200            |
| 3   | 16                                          | 0                 | 64                | 1.950                    | 2.100            |
| 4   | 24                                          | 0                 | 56                | 1.527                    | 2.000            |
| 5   | 32                                          | 0                 | 48                | 1.490                    | 2.200            |
| 6   | 40                                          | 0                 | 40                | 1.558                    | 2.408            |
| 7   | 48                                          | 0                 | 32                | 1.795                    | 2.328            |
| 8   | 56                                          | 0                 | 24                | 1.540                    | 2.400            |
| 9   | 64                                          | 0                 | 16                | 1.444                    | 2.220            |
| 10  | 72                                          | 0                 | 8                 | 1.400                    | 2.100            |
| 11  | 80                                          | 0                 | 0                 | 1.100                    | 1.150            |

The total growth of the 11 cultures, without creatinine, composed of mixtures of phosphate and potash was 16,674 grams against 22,682 grams for the cultures with creatinine. This is an increase of 36 per cent in the creatinine cultures.

The effect of creatinine in cultures with no nitrate is shown in Plate II. Cultures marked with the same number, for instance 1 and 1c, have similar fertilizer ratios. The cultures marked with numbers alone have no creatinine, the numbers with the letter *c* have 50 parts per million of creatinine. Culture No. 1 is composed of potash only, 80 parts per million; No. 2 has 72 parts per million of potash and 8 parts per million of phosphate; No. 3 has 64 parts per million of potash and 16 parts per million of phosphate; No. 4 has 56 parts per million of potash and 24 parts per million of phosphate; No. 5 has 48 parts per million of potash and 32 parts per million of phosphate; No. 6 has 40 parts per million of potash and 40 parts per million of phosphate; No. 7 has 32 parts per million of potash and 48 parts per million of phosphate; No. 8 has 24 parts per million of potash and 56 parts per million of phosphate; No. 9 has 16 parts per million of potash and 64 parts per million of phosphate; No. 10 has 8 parts per million of potash and 72 parts per million of phosphate; and No. 11 is composed of phosphate only, 80 parts per million.

As shown in the photograph, the plants in each culture containing creatinine, no matter what the proportion of potash and phosphate, is larger than the plants grown in a similar solution without the creatinine. The increased growth is noticeable in the roots as well as in the tops. The tops in each case are broader and taller and the roots are larger and better branched.

CREATININE IN CULTURES CONTAINING EIGHT PARTS PER MILLION  $\text{NH}_3$  AS NITRATE.

Since creatinine was very beneficial in cultures containing no nitrate it is interesting to observe its effect in cultures which contain a small amount of nitrate. Table II gives the result of the effect of creatinine on growth in culture solutions composed of eight parts per million of  $\text{NH}_3$  as nitrate and varying amounts of phosphate and potash, the total concentration of each solution being 80 parts per million of  $\text{P}_2\text{O}_5$ ,  $\text{NH}_3$ , and  $\text{K}_2\text{O}$ . By comparing the figures in the second and third columns it is seen that the growth with creatinine, given in the last column, is larger than the growth without creatinine, given in the fifth column. The difference, however, is not near so great as in solutions containing no nitrate, presented in Table I. The total green weight of the cultures composed of fertilizer mixtures containing 8 parts per million of  $\text{NH}_3$  without creatinine was 24.071 grams against 28.117 grams in the cultures with creatinine, an increase of 17 per cent. In the cultures with no nitrate creatinine produced an increase of 36 per cent.

TABLE II.—*Effect of creatinine on growth in culture solutions composed of fertilizer mixtures, containing 8 parts per million of  $\text{NH}_3$ , as nitrate.*

| No. | Fertilizer ingredients in culture solution. |                 |                        | Green weight of culture. |                  |
|-----|---------------------------------------------|-----------------|------------------------|--------------------------|------------------|
|     | $\text{P}_2\text{O}_5$ .                    | $\text{NH}_3$ . | $\text{K}_2\text{O}$ . | Without creatinine.      | With creatinine. |
|     | <i>P. p. m.</i>                             | <i>P. p. m.</i> | <i>P. p. m.</i>        | <i>Grams.</i>            | <i>Grams.</i>    |
| 1   | 0                                           | 8               | 72                     | 1.820                    | 2.190            |
| 2   | 8                                           | 8               | 64                     | 2.470                    | 3.100            |
| 3   | 16                                          | 8               | 56                     | 2.748                    | 3.250            |
| 4   | 24                                          | 8               | 48                     | 2.907                    | 3.420            |
| 5   | 32                                          | 8               | 40                     | 2.670                    | 2.450            |
| 6   | 40                                          | 8               | 32                     | 2.928                    | 3.258            |
| 7   | 48                                          | 8               | 24                     | 2.526                    | 3.340            |
| 8   | 56                                          | 8               | 16                     | 2.600                    | 3.000            |
| 9   | 64                                          | 8               | 8                      | 2.048                    | 2.359            |
| 10  | 72                                          | 8               | 0                      | 1.354                    | 1.750            |

CREATININE IN CULTURES WITH LARGER AMOUNTS OF NITRATE.

It has been shown that creatinine was very beneficial in cultures which contained no nitrate. In a group of cultures, composed of mixtures of phosphate and potash in different proportions, creatinine increased the growth 36 per cent. It has also been pointed out that the beneficial effect of creatinine was not so great in cultures containing a small amount of nitrogen. In the group of cultures composed of mixtures of potash, phosphate, and 8 parts per million of  $\text{NH}_3$  as nitrate creatinine increased the growth only 17 per cent.

In Table III are given the results of growth in cultures with and without creatinine, composed of mixtures of phosphate, potash, and nitrate. The proportion of phosphate and potash vary in the



different cultures, but each solution has 16 parts per million of  $\text{NH}_3$  as nitrate. The green weights of the creatinine cultures, given in the last column of the table, are slightly larger than the control cultures shown in the fourth column. The total green weight of the cultures without creatinine was 25.516 grams against 27.573 grams for the cultures with creatinine, an increase of 8 per cent.

TABLE III.—*Effect of creatinine in cultures containing 16 parts per million of  $\text{NH}_3$  as nitrate.*

| No. | Fertilizer ingredients in culture solution. |                 |                        | Green weight of cultures. |                  |
|-----|---------------------------------------------|-----------------|------------------------|---------------------------|------------------|
|     | $\text{P}_2\text{O}_5$ .                    | $\text{NH}_3$ . | $\text{K}_2\text{O}$ . | Without creatinine.       | With creatinine. |
|     | <i>P. p. m.</i>                             | <i>P. p. m.</i> | <i>P. p. m.</i>        | <i>Grams.</i>             | <i>Grams.</i>    |
| 1   | 0                                           | 16              | 64                     | 2.200                     | 2.570            |
| 2   | 8                                           | 16              | 56                     | 3.200                     | 3.720            |
| 3   | 16                                          | 16              | 48                     | 3.400                     | 3.500            |
| 4   | 24                                          | 16              | 40                     | 3.097                     | 3.702            |
| 5   | 32                                          | 16              | 32                     | 3.250                     | 3.250            |
| 6   | 40                                          | 16              | 24                     | 3.228                     | 3.300            |
| 7   | 48                                          | 16              | 16                     | 2.975                     | 3.240            |
| 8   | 56                                          | 16              | 8                      | 2.626                     | 2.551            |
| 9   | 64                                          | 16              | 0                      | 1.440                     | 1.740            |

In other cultures composed of the three fertilizer ingredients,  $\text{P}_2\text{O}_5$ ,  $\text{NH}_3$ , and  $\text{K}_2\text{O}$ , but containing 24 parts per million  $\text{NH}_3$  as nitrate, creatinine only increased growth 2 per cent. Its effect in cultures composed of fertilizer mixtures having more than 24 parts per million of  $\text{NH}_3$  as nitrate was uncertain. In some cases there was a slight increase in growth and in others there was a slight decrease. However, in all cases with large amounts of nitrate the growth was practically the same in the normal and creatinine cultures.

Before discussing further the effect of creatinine it will be necessary to show the effect which nitrates have on the growth of plants in mixtures of the other two fertilizer ingredients, potash and phosphate. In work previously published <sup>1</sup> it was shown that the better growth occurred in the normal cultures, when the three fertilizer elements  $\text{P}_2\text{O}_5$ ,  $\text{NH}_3$ , and  $\text{K}_2\text{O}$  were present, and was best in mixtures which contained approximately equal amounts of  $\text{NH}_3$  and  $\text{K}_2\text{O}$  and a small amount of  $\text{P}_2\text{O}_5$ , about 16 parts per million. The growth in the cultures containing the three constituents was much greater than in cultures containing only two constituents. This was especially marked when nitrate was not in the composition. In illustration of this the average growth of a number of cultures, composed of mixtures of phosphate and potash, in amounts of 80 parts per million of  $\text{P}_2\text{O}_5 + \text{K}_2\text{O}$ , was 1.000 gram against 3.155 grams as the average growth of cultures composed of mixtures of these two

<sup>1</sup> Schreiner, O., and Skinner, J. J. Some Effects of a Harmful Organic Soil Constituent. Bul. 70, Bureau of Soils, U. S. Dept. of Agr., 1910.

ingredients, with an addition of only 8 parts per million of  $\text{NH}_3$  as nitrate, the total concentration of nutrients being the same. In a second experiment conducted in a similar manner, but at a later date, the average growth in the cultures composed of mixtures of phosphate and potash was 0.878 gram and the average growth in cultures consisting of mixtures of the three ingredients, 8 parts per million of the mixture being  $\text{NH}_3$ , was 2.107 grams.

In the present experiment the growth in the normal cultures composed of varying proportions of phosphate and potash, compared with the growth in mixtures of these two ingredients, with 8 parts per million of  $\text{NH}_3$  added, is given in Table IV.

TABLE IV.—*Showing the growth of cultures, composed of fertilizer mixtures containing no nitrate, and 8 parts per million of  $\text{NH}_3$  as nitrate; without creatinine.*

| No. | Fertilizer ingredients in culture solution. |                 |                        | Green weight of cultures. |                |
|-----|---------------------------------------------|-----------------|------------------------|---------------------------|----------------|
|     | $\text{P}_2\text{O}_5$ .                    | $\text{NH}_3$ . | $\text{K}_2\text{O}$ . | No nitrogen.              | With nitrogen. |
|     | <i>P. p. m.</i>                             | <i>P. p. m.</i> | <i>P. p. m.</i>        | <i>Grams.</i>             | <i>Grams.</i>  |
| 1   | 0                                           | 0               | 80                     | 1.400                     | 1.820          |
| 2   | 0                                           | 8               | 72                     | 1.470                     | 2.470          |
| 3   | 8                                           | 0               | 64                     | 1.950                     | 2.748          |
| 4   | 8                                           | 8               | 56                     | 1.527                     | 2.907          |
| 5   | 16                                          | 0               | 48                     | 1.490                     | 2.670          |
| 6   | 16                                          | 8               | 40                     | 1.558                     | 2.928          |
| 7   | 24                                          | 0               | 32                     | 1.795                     | 2.526          |
| 8   | 24                                          | 8               | 24                     | 1.540                     | 2.600          |
| 9   | 32                                          | 0               | 16                     | 1.444                     | 2.048          |
| 10  | 32                                          | 8               | 8                      | 1.400                     | 1.354          |
| 11  | 40                                          | 0               | 0                      | 1.100                     |                |
| 12  | 40                                          | 8               |                        |                           |                |
| 13  | 48                                          | 0               |                        |                           |                |
| 14  | 48                                          | 8               |                        |                           |                |
| 15  | 56                                          | 0               |                        |                           |                |
| 16  | 56                                          | 8               |                        |                           |                |
| 17  | 64                                          | 0               |                        |                           |                |
| 18  | 64                                          | 8               |                        |                           |                |
| 19  | 72                                          | 0               |                        |                           |                |
| 20  | 72                                          | 8               |                        |                           |                |
| 21  | 80                                          | 0               |                        |                           |                |

The growth of culture No. 11, for instance, composed of 40 parts per million of  $\text{P}_2\text{O}_5$  and 40 parts per million  $\text{K}_2\text{O}$ , is 1.558 grams against 2.670 grams for culture No. 10, which has 32 parts per million of  $\text{P}_2\text{O}_5$ , 40 parts per million  $\text{K}_2\text{O}$ , and 8 parts per million  $\text{NH}_3$ , or 2.928 grams for culture No. 12, which has 40 parts per million of  $\text{P}_2\text{O}_5$ , 32 parts per million of  $\text{K}_2\text{O}$ , and 8 parts per million of  $\text{NH}_3$ . By a close examination of the figures in this table it is seen that the growth in the mixtures of phosphate and potash is smaller than in cultures composed of mixtures of the three ingredients, containing 8 parts per million of  $\text{NH}_3$  as nitrate. The average growth of the cultures without nitrate is 1.516 grams against 2.407 grams with 8 parts per million of  $\text{NH}_3$  in the fertilizer mixture. Putting the growth of the cultures without nitrate at 100, the relative growth of the cultures with nitrate becomes 159, or an increase of 59 per cent.



In Table V are given the green weights of cultures with creatinine in the solution, similar to the results discussed above with the normal cultures. With 50 parts per million of creatinine in the solution the cultures containing no nitrate produced better growth than the corresponding cultures without creatinine shown in Table IV. The difference between the two last columns is not as marked in the creatinine set as in the normal set. For instance, culture No. 11, containing 40 parts per million each of phosphate and potash, produced 2.408 grams green weight against 2.450 grams for culture No. 10, which has 32 parts per million of phosphate, 40 parts per million of potash, and 8 parts per million of  $\text{NH}_3$  as nitrate, and 3.258 grams for culture No. 12, which has 40 parts per million of phosphate, 32 parts per million of potash, and 8 parts per million of  $\text{NH}_3$  as nitrate. The average growth of the cultures without nitrate is 2.062 grams against 2.812 grams for each culture having 8 parts per million of  $\text{NH}_3$  as nitrate in the fertilizer mixture. If the growth of the cultures without nitrate is put at 100, the growth with nitrate in the fertilizer mixture becomes 136, or an increase of 36 per cent.

TABLE V.—*Growth of cultures composed of fertilizer mixtures containing no nitrate, and 8 parts per million of  $\text{NH}_3$  as nitrate; with creatinine.*

| No. | Fertilizer ingredients in culture solution. |                 |                        | Green weight of cultures. |                |
|-----|---------------------------------------------|-----------------|------------------------|---------------------------|----------------|
|     | $\text{P}_2\text{O}_5$ .                    | $\text{NH}_3$ . | $\text{K}_2\text{O}$ . | No nitrogen.              | With nitrogen. |
|     | <i>P. p. m.</i>                             | <i>P. p. m.</i> | <i>P. p. m.</i>        | <i>Grams.</i>             | <i>Grams.</i>  |
| 1   | 0                                           | 0               | 80                     | 1.576                     | .....          |
| 2   | 0                                           | 8               | 72                     | .....                     | 2.190          |
| 3   | 8                                           | 0               | 72                     | 2.200                     | .....          |
| 4   | 8                                           | 8               | 64                     | .....                     | 3.100          |
| 5   | 16                                          | 0               | 64                     | 2.100                     | .....          |
| 6   | 16                                          | 8               | 56                     | .....                     | 3.250          |
| 7   | 24                                          | 0               | 56                     | 2.000                     | .....          |
| 8   | 24                                          | 8               | 48                     | .....                     | 3.420          |
| 9   | 32                                          | 0               | 48                     | 2.200                     | .....          |
| 10  | 32                                          | 8               | 40                     | .....                     | 2.450          |
| 11  | 40                                          | 0               | 40                     | 2.408                     | .....          |
| 12  | 40                                          | 8               | 32                     | .....                     | 3.258          |
| 13  | 48                                          | 0               | 32                     | 2.328                     | .....          |
| 14  | 48                                          | 8               | 24                     | .....                     | 3.340          |
| 15  | 56                                          | 0               | 24                     | 2.400                     | .....          |
| 16  | 56                                          | 8               | 16                     | .....                     | 3.000          |
| 17  | 64                                          | 0               | 16                     | 2.220                     | .....          |
| 18  | 64                                          | 8               | 8                      | .....                     | 2.359          |
| 19  | 72                                          | 0               | 8                      | 2.100                     | .....          |
| 20  | 72                                          | 8               | 0                      | .....                     | 1.750          |
| 21  | 80                                          | 0               | 0                      | 1.150                     | .....          |

In the control set of cultures without creatinine, presented in Table IV, the relative growth in solutions with nitrogen was 59 per cent greater than the growth in the cultures having no nitrogen in the fertilizer mixture. In the cultures with creatinine there was a difference of only 36 per cent in growth between the no-nitrate and the nitrate cultures. This might be taken to indicate that the creatinine replaced the efficiency of nitrate in producing plant growth.

Plate III shows the effect of creatinine with and without nitrate. As seen in the photograph, there is a large difference between the control cultures having a small amount of nitrate (series A) and those without nitrate (series B). The no-nitrate plants have small tops and poorly developed roots, while the nitrate plants have large tops and well branched roots. Series C, creatinine cultures without nitrate, compared with series D, creatinine cultures with nitrate, shows that this difference is not so great as in the case of the normal cultures with and without nitrate.

The effect of creatinine in cultures containing no nitrate is well shown by comparing series B with series C. Series A compared with series D shows that creatinine does not produce so great an additional effect when nitrogen as nitrate is in the culture.

#### INFLUENCE OF CREATININE ON ABSORPTION OF FERTILIZER SALTS.

The foregoing discussion has shown clearly the influence of creatinine on growth and its effect in cultures containing no nitrate. There remains to be discussed the effect of the creatinine on the removal of nutrients from the solution during the growth of the plant.

Mention has already been made of the fact that the concentration differences produced by the growth of the plants in the various cultures was determined by making an analysis for nitrate at the termination of every three-day change, and of the phosphate and potassium on a composite of the solutions from the four changes. It is thus possible to compare the results obtained under the so-called normal conditions without the creatinine and under the conditions where 50 parts per million of creatinine were present in the solution.

The sum total of  $P_2O_5$ ,  $NH_3$ , and  $K_2O$  removed from solution by the growing plants in the cultures containing all three of these constituents was 1,684 milligrams under the normal conditions and 1,584 milligrams in the creatinine set. The figures show the total of plant nutrients removed to be slightly less in the creatinine set, although the green weight in this set was 9 per cent greater than in the normal set. The examination of the results for the three constituents separately, as given below, shows that the phosphate and potash absorption were slightly greater than normal, as is demanded by the larger growth, whereas the nitrate removal is considerably less than in the normal set.

*Phosphate.*—The phosphate in the cultures was determined by the method described on page 59 of Bulletin 70, Bureau of Soils. The amount of phosphate stated as  $P_2O_5$  removed from the total number of solutions during the experiment was 364 milligrams for the normal cultures and 383 milligrams for the cultures containing creatinine, a difference of 19 milligrams in favor of the creatinine cultures.

*Potassium.*—The potassium was determined on a composite of the four changes by a method previously described.<sup>1</sup> The amount of potash stated as  $K_2O$  removed by the plants in the total number of cultures was 760 milligrams in the case of the normal cultures and 778 milligrams for the cultures with creatinine. As with the phosphate, the creatinine cultures removed a little more potash than the normal cultures, there being a difference of 18 milligrams in favor of the creatinine set.

*Nitrates.*—The solutions were analyzed for nitrates at the end of each three-day change, similar to the methods described in Bulletin 70, Bureau of Soils. The total amount of nitrogen stated as  $NH_3$  removed from the total number of solutions during the course of the experiment was 560 milligrams for the normal cultures and 423 milligrams for the creatinine cultures. The creatinine cultures, although making a larger growth, used 137 milligrams less nitrate.

#### EFFECT OF CREATINE ON GROWTH.

Experiments in nutrient cultures with creatine have been conducted similar to those with creatinine. Creatine is closely related chemically to creatinine. Both probably occur in soils, manures, and green crops, a discussion of which is given in the two other papers in this bulletin.

The action of creatine on growth was determined by growing wheat seedlings in nutrient solutions of calcium acid phosphate, sodium nitrate, and potassium sulphate, similar to the method described earlier in this paper. The concentration of the solution was 80 parts per million of  $P_2O_5 + NH_3 + K_2O$ . The plants grew from April 22 to May 4. Notes were taken during the period of growth, and the green weight recorded at the end of the experiment. The culture solutions were changed every three days and the solutions analyzed for nitrate, phosphate, and potash.

After the plants had grown for several days it was apparent that the effect of creatine was very similar to that of creatinine. The leaves were broader and further developed than the control cultures. The roots were longer and better branched. The plants growing in creatine cultures which contained phosphate and potash but no nitrogen were a great deal larger than similar cultures without creatine. Like the creatinine, when a small amount of nitrate was in the fertilizer mixture the beneficial effect of creatine was not so marked, and in the presence of a larger amount of nitrate creatine had no additional effect.

The total green weight of 66 cultures, containing the fertilizer salts only—that is, the normal set—was 174.4 grams, against 186.8 grams

<sup>1</sup> Schreiner, O., and Failyer, G. H. Colorimetric, Turbidity, and Titration Methods Used in Soil Investigations. Bul. 31, Bureau of Soils, U. S. Dept. Agr. (1906).



for the 66 cultures containing 50 parts per million of creatine in addition to the fertilizer salts. This is an increase for the creatine cultures of 8 per cent over the normal cultures.

Table VI shows the effect of creatine on growth in a number of cultures containing varying amounts of phosphate and potash, but no nitrate, the amount of total fertilizer ingredient in each culture being 80 parts per million. From an examination of the table it is apparent that the growth of each of the creatine cultures, given in the last column, is considerably larger than the growth of the cultures without creatine, given in the fifth column. Culture No. 3, which has 16 parts per million of  $P_2O_5$  and 64 parts per million of  $K_2O$ , produced 2.130 grams green weight with creatine and 1.558 grams without creatine. Culture No. 6, which has equal amounts of  $P_2O_5$  and  $K_2O$ , produced 2.400 grams green weight with creatine and 1.5 grams without this substance. Culture No. 9, which has 64 parts per million of  $P_2O_5$  and 16 parts per million of  $K_2O$ , produced 2.450 grams green weight with creatine and 1.600 grams without creatine. The total green weight of the cultures without creatine was 16.2 grams against 23.3 grams for the cultures with creatine, an increase of 44 per cent.

TABLE VI.—*Effect of creatine on growth in cultures containing no nitrate.*

| No. | Fertilizer ingredients in culture solution. |                 |                 | Green weight of cultures. |                |
|-----|---------------------------------------------|-----------------|-----------------|---------------------------|----------------|
|     | $P_2O_5$ .                                  | $NH_3$ .        | $K_2O$ .        | Without creatine.         | With creatine. |
|     | <i>P. p. m.</i>                             | <i>P. p. m.</i> | <i>P. p. m.</i> | <i>Grams.</i>             | <i>Grams.</i>  |
| 1   | 0                                           | 0               | 80              | 1.329                     | 1.709          |
| 2   | 8                                           | 0               | 72              | 1.420                     | 1.948          |
| 3   | 16                                          | 0               | 64              | 1.558                     | 2.130          |
| 4   | 24                                          | 0               | 56              | 1.579                     | 2.370          |
| 5   | 32                                          | 0               | 48              | 1.528                     | 2.470          |
| 6   | 40                                          | 0               | 40              | 1.500                     | 2.400          |
| 7   | 48                                          | 0               | 32              | 1.670                     | 2.270          |
| 8   | 56                                          | 0               | 24              | 1.628                     | 2.420          |
| 9   | 64                                          | 0               | 16              | 1.600                     | 2.450          |
| 10  | 72                                          | 0               | 8               | 1.428                     | 2.070          |
| 11  | 80                                          | 0               | 0               | .978                      | 1.090          |

In Table VII are given the green weights of plants grown in cultures with and without creatine, containing 8 parts per million of  $NH_3$  as nitrate and varying amounts of  $P_2O_5$  and  $K_2O$ , the total constituents being 80 parts per million of  $P_2O_5 + NH_3 + K_2O$ . These figures show that the creatine cultures, given in the last column, are somewhat larger than the cultures without creatine, given in the fifth column, but the difference is not near so large as in the cultures containing no nitrate given in Table VI. The total growth of the cultures without creatine was 26.4 grams against 29.4 grams for the cultures with creatine, an increase of only 11 per cent in favor of the creatine cultures. There was a difference of 44 per cent in favor



of the creatine cultures, in the case of the solutions which contained no nitrate.

TABLE VII.—*Effect of creatine on growth in cultures containing 8 parts per million of  $\text{NH}_3$  as nitrate.*

| No. | Fertilizer ingredients in culture solution. |                 |                        | Green weight of cultures. |                   |
|-----|---------------------------------------------|-----------------|------------------------|---------------------------|-------------------|
|     | $\text{P}_2\text{O}_5$ .                    | $\text{NH}_3$ . | $\text{K}_2\text{O}$ . | Without creatine.         | With creatine.    |
|     | <i>P. p. m.</i>                             | <i>P. p. m.</i> | <i>P. p. m.</i>        | <i>Grams.</i>             | <i>Grams.</i>     |
| 1   | 0                                           | 8               | 72                     | 2.299                     | 2.459             |
| 2   | 8                                           | 8               | 64                     | 2.940                     | 3.200             |
| 3   | 16                                          | 8               | 56                     | 2.700                     | 3.350             |
| 4   | 24                                          | 8               | 48                     | 2.920                     | 3.400             |
| 5   | 32                                          | 8               | 40                     | 3.050                     | 3.07 <sup>^</sup> |
| 6   | 40                                          | 8               | 32                     | 3.150                     | 3.309             |
| 7   | 48                                          | 8               | 24                     | 3.220                     | 3.350             |
| 8   | 56                                          | 8               | 16                     | 2.500                     | 2.854             |
| 9   | 64                                          | 8               | 8                      | 2.222                     | 2.800             |
| 10  | 72                                          | 8               | 0                      | 1.400                     | 7.600             |

The growth in cultures which contained varying amounts of phosphate and potash and 16 parts per million of  $\text{NH}_3$  as nitrate was only 3 per cent greater with creatine than without creatine. In solutions containing 24 parts per million of  $\text{NH}_3$  as nitrate the increased growth with creatine was 6 per cent, and in solutions containing 32 parts per million  $\text{NH}_3$  as nitrate the increased growth was 4 per cent. In solutions containing higher amounts of nitrate the creatine had no additional effect. Thus it appears that the effect of creatine in replacing the effect of nitrate in producing growth is very similar to that of creatinine.

It is also interesting to note the effect of creatine on the removal of salts by the plants, and the similarity between the action of creatine and creatinine in this respect. It will be remembered that in the creatinine cultures the removal of phosphate and potash was slightly greater in the creatinine cultures than in the normal cultures, but a great deal less nitrate disappeared from solution in the creatinine than in the normal cultures.

In the creatine experiment the removal of total  $\text{P}_2\text{O}_5$ ,  $\text{NH}_3$ , and  $\text{K}_2\text{O}$  by plants in the control cultures was 1,978.3 milligrams, against 1,854.5 milligrams for the creatine cultures. The control cultures removed 471.0 milligrams of phosphate, stated as  $\text{P}_2\text{O}_5$ , and the creatine cultures 474.4 milligrams. In the case of potash the control cultures removed 769.4 milligrams of  $\text{K}_2\text{O}$  against 767.4 milligrams for the creatine cultures. The removal of both phosphate and potash was practically the same in the control and creatine cultures. The disappearance of nitrate was much less in the creatine than in the control cultures. The control cultures removed 737.7 milligrams, against 612.7 milligrams for the creatine cultures, a difference of 125 milligrams.

The influence of the creatine as regards the removal of  $P_2O_5$ ,  $NH_3$ , and  $K_2O$  is very similar to that shown by creatinine, and it again appears that this substance as well as the creatinine can replace nitrate in its effects on plant growth.

#### SUMMARY.

Creatinine and creatine have beneficial effects on plant growth.

Plants grown in solution cultures containing only potash and phosphate show greatly increased growth when creatinine or creatine is added.

When large amounts of nitrate are present in the culture solutions, creatinine or creatine produce no appreciable effect on the growth.

Plants growing in cultures, whether low or high in nitrate, showed a greatly diminished absorption of this ingredient when creatinine or creatine was present, whereas the removal of potash and phosphate was practically normal.

It appears, therefore, that creatinine and creatine can replace the effect of nitrate in plant growth.

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